

EXHIBIT A

Yeast Sml1, a Protein Inhibitor of Ribonucleotide Reductase*

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Ribonucleotide reductase (RNR) catalyzes the reduction of ribonucleotides to deoxyribonucleotides; this step is rate-limiting in DNA precursor synthesis. A number of regulatory mechanisms ensure optimal deoxyribonucleotide pools, which are essential for cell viability. The best studied mechanisms are transcriptional regulation of the RNR genes during the cell cycle and in the response to DNA damage, and the allosteric regulation of ribonucleotide reductase by nucleoside triphosphates. Recently, another mode of RNR regulation has been hypothesized in yeast. A novel protein, Sml1, was shown to bind to the Rnr1 protein of the yeast ribonucleotide reductase; this interaction was proposed to inhibit ribonucleotide reductase activity when DNA synthesis is not required (Zhao, X., Muller, E.G.D., and Rothstein, R. (1998) *Mol. Cell* 2, 329–340). Here, we use highly purified recombinant proteins to directly demonstrate that the Sml1 protein is a strong inhibitor of yeast RNR. The Sml1p specifically binds to the yeast Rnr1p in a 1:1 ratio with a dissociation constant of 0.4 μ M. Interestingly, Sml1p also specifically binds to the mouse ribonucleotide reductase R1 protein. However, the inhibition observed in an *in vitro* mouse ribonucleotide reductase assay is less pronounced than the inhibition in yeast and probably occurs via a different mechanism.

Ribonucleotide reductase (RNR)¹ plays a crucial role in DNA synthesis, by catalyzing the direct reduction of all four ribonucleotides to deoxyribonucleotides. Both the yeast and mammalian ribonucleotide reductases belong to the ribonucleotide reductase class Ia; in this class, the active enzymes consist of a large subunit and a small one (1). In mammalian cells, these two non-identical homodimeric subunits are encoded by the R1 and R2 genes, respectively. The R1 protein contains redox-active dithiols, the active site binding nucleoside diphosphate substrates and binding sites for nucleoside triphosphates that act as allosteric effectors. Substrate specificity is controlled by binding of ATP, dATP, dTTP, or dGTP to a specificity site, whereas overall activity is controlled by binding of ATP (active) or dATP (inactive) to an activity site (2). Failure to control the size of dNTP pools and/or their relative amounts leads to cell death or genetic abnormalities (3).

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¹ The abbreviations used are: RNR, ribonucleotide reductase; RU, resonance unit; PAGE, polyacrylamide gel electrophoresis.

The R2 protein contributes a tyrosyl-free radical, which is essential for RNR activity; this radical is generated by a binuclear iron center (2). The flexible C-terminal tail of the R2 polypeptide chain is essential for the R1 and R2 interaction, and upon binding to the R1 protein, the flexible R2 protein tail becomes rigid (4). Peptides and peptidomimetics corresponding to the R2 protein C-terminal inhibit ribonucleotide reductase in a species specific way (5).

In *Saccharomyces cerevisiae*, there are two genes encoding R1-like proteins, *RNR1* and *RNR3* (6); there are also two genes encoding R2-like proteins, *RNR2* (7, 8) and *RNR4* (9, 10). The yeast ribonucleotide reductase genes are one of the targets of the Mec1-Rad53-dependent DNA damage/cell cycle checkpoint pathway (11). Overexpression of the yeast RNR genes suppresses the lethality of strains lacking Mec1 or Rad53, supposedly by increasing the dNTP pools (12). Unlike the mammalian enzyme, the yeast RNR is not inhibited by physiological concentrations of dATP; this observation explains the positive correlation between enzyme and dNTP levels in yeast.² Recently, Zhao *et al.* (13) identified a novel protein that negatively affects dNTP pools in yeast, and they called it Sml1p. Deletion of *SML1* rescued the lethality of a *mec1* or *rad53* strain. Sml1p was shown to interact with the yeast Rnr1 protein using the two-hybrid system and co-immunoprecipitations. For this reason, a novel mode of RNR regulation was suggested, where Sml1p binding to the Rnr1 protein would inhibit the enzyme.

Using highly purified recombinant proteins, we now directly demonstrate in an *in vitro* yeast ribonucleotide reductase assay that Sml1p is indeed a very potent inhibitor of yeast RNR. It specifically binds to the yeast Rnr1p as shown by biosensor technique using sensor chips with immobilized Sml1p. Interestingly, the Sml1p also specifically binds to the mouse R1 protein with high affinity. However, the inhibition seen in an *in vitro* mouse ribonucleotide reductase assay is less pronounced than in the yeast system, which indicates an inhibition mechanism different from the one in yeast. These findings may be used to develop a new generation of antiproliferative drugs targeted to RNR.

MATERIALS AND METHODS

Recombinant Proteins and Peptides—The recombinant yeast proteins Rnr1, Rnr2, and Rnr4 were expressed in *Escherichia coli* BL21(DE3) bacteria using the pET3a expression vector; mouse recombinant proteins R1 and R2 were expressed in *E. coli* BL21(DE3)pLysS bacteria using the same vector (14). Purification of the recombinant mouse and yeast R1 proteins, and of the recombinant mouse R2 protein, was made as described earlier (15, 16). The yeast Rnr2 and Rnr4 proteins were coexpressed and purified as a heterodimer.³ The *SML1* coding sequence (13) was amplified by polymerase chain reaction from yeast genomic DNA using the following oligonucleotides: 5'-CAA TAA TTT CCC CAT ATG CAA AAT TCC-3' and 5'-AAA GGA TCC TTA GAA GTC CAT TTC CTC GAC-3'. After the polymerase chain reaction product was cleaved with *Nde*I and *Bam*HI restriction endonucleases, it was

² A. Chabes and L. Thelander, submitted for publication.

³ V. Domkin, A. Chabes, and L. Thelander, manuscript in preparation.

cloned into the pET3a vector digested with the same restriction enzymes. The *Sml1* sequence in the resulting plasmid was checked by DNA sequencing. Recombinant yeast *Sml1p* was expressed in *E. coli* BL21(DE3)pLysS bacteria by growing a culture at 37 °C to an optical density of 0.6 at 600 nm, followed by induction with 0.5 mM isopropyl-1-thio- β -D-galactopyranoside. After 3 h, the bacteria were harvested by centrifugation, resuspended in 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, and lysed by freezing in liquid nitrogen. After thawing, the lysate was centrifuged at $150,000 \times g$, 4 °C for 1 h. Proteins in the supernatant were precipitated by adding solid ammonium sulfate to 25% saturation at 0 °C (0.136 g/ml). The pellet was dissolved in the same buffer as above; finally, the solution was centrifuged through Ultrafree-MC Millipore 30,000 NMWL filter units with a 30-kDa cut-off value to obtain pure *Sml1* protein in the filtrate.

N-acetylated peptides corresponding to the last 9 amino acids of either Rnr2p (GAFTFNEDF), Rnr4p (KEINFDDDF), or *Sml1p* (QKGVEEMDF) were ordered from Genosys.

Protein Concentrations—Protein concentrations were determined by reading the absorbance at 280 nm, and using the earlier published $E^{1\%}_{1\text{cm}}$ of 12 (mouse R1 and yeast Rnr1 proteins; Ref. 15) or 13.7 (mouse R2 protein; Ref. 16). The corresponding value for the Rnr2p/Rnr4p heterodimer (12.9) was obtained from quantitative amino acid hydrolysis of aliquots from solutions with known absorbance. The concentration of *Sml1p* solutions was determined using an $E^{1\%}_{1\text{cm}}$ of 7.1 based on calculations (DNASTar, Inc.) from the amino acid composition.

BLAcore Biosensor Analysis—The interaction between *Sml1p* and either yeast Rnr1p or mouse R1 protein was studied by biosensor analysis using the BLAcore method (Biacore AB). The *Sml1* protein was prepared in 10 mM sodium acetate, pH 5.2, at a concentration of 0.44 mg/ml; it was then immobilized on the dextran layer of the sensor chip as described previously for the mouse R2 protein (17). The ligand proteins were equilibrated with running buffer (10 mM Hepes-KOH, pH 7.4, 200 mM potassium acetate, 1 mM EDTA, 5 mM magnesium acetate, and 0.05% Surfactant P20 (BLAcore)). The interactions were studied at a constant temperature of 22 °C and a constant flow of 5 μ l/min. Kinetics for the interaction of *Sml1p* with yeast Rnr1p was determined by allowing the immobilized *Sml1p* to interact with increasing concentrations of Rnr1p (18). The resonance unit (RU) is proportional to the mass, and 1 RU corresponds to a surface concentration of 1 pg of protein/mm² of the 100-nm thick dextran layer (19).

Sucrose Gradient Centrifugation—Rnr1p (500 μ g) was incubated in 0.1 ml of 20 mM Hepes-KOH, pH 7.4, 100 mM potassium acetate, 10 mM magnesium acetate, 5 mM dithiothreitol, and 0.1 mM dTTP if indicated, in the presence or absence of 90 μ g of *Sml1* protein. After 30 min of incubation at 25 °C, the samples were cooled on ice and mixed with 0.75 μ g of bovine liver catalase (Sigma); catalase served as a marker for the determination of sedimentation coefficients. The 0.1-ml samples were layered onto 4.0 ml of a 5–20% linear gradient of sucrose in the same buffer as above, and centrifuged for 14 h at 40,000 rpm in a Beckman SW 60 rotor at 4 °C. The bottom of each tube was punctured, and fractions of approximately 0.1 ml were collected. Aliquots of each fraction were removed for spectrophotometric assay of catalase activity (20), protein concentration as determined by the Bradford method (21), or SDS-PAGE analysis.

RESULTS

Purification of Recombinant Yeast *Sml1* Protein—As seen in Fig. 1, pure recombinant *Sml1* protein was obtained after only three purification steps: ultracentrifugation, ammonium sulfate fractionation, and ultrafiltration. The yield was about 1 mg of pure protein/liter of bacterial culture.

Inhibition of Yeast Ribonucleotide Reductase by the *Sml1* Protein—Recombinant yeast ribonucleotide reductase was assayed for activity using a CDP reduction assay, with ATP as a positive effector in the presence of increasing amounts of *Sml1* protein (Fig. 2). A molar ratio of 1:1 between Rnr1p monomer (M_r 100,000) and *Sml1p* (M_r 11,800) gave about 50% inhibition. The amounts of Rnr2p/Rnr4p heterodimer were varied (1–32 μ g) at one fixed amount of Rnr1p (2 μ g) and 0, 0.2, and 2 μ g of *Sml1p*; this experiment did not give any evidence of competition between *Sml1p* and the Rnr2p/Rnr4p heterodimer for binding to Rnr1p. Instead the data fitted best to a noncompetitive inhibition (data not shown).

Inhibition of Yeast Ribonucleotide Reductase by Nonapeptides Corresponding to the C-terminal Ends of *Sml1p*, Rnr2p, or

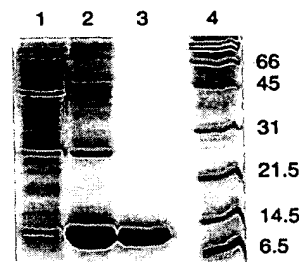


FIG. 1. Samples of *Sml1p* preparations after different purification steps analyzed on a 15% SDS-PAGE and stained by Coomassie Brilliant Blue. Lane 1, 10 μ g of protein lysate after the ultracentrifugation; lane 2, 10 μ g of protein after the ammonium sulfate fractionation; lane 3, 10 μ g of protein after the ultrafiltration, lane 4, molecular weight markers (kDa).

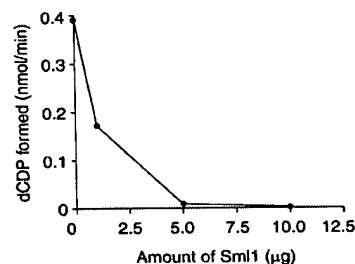


FIG. 2. Inhibition of yeast RNR by *Sml1p*. A series of tubes containing yeast RNR (10 μ g of Rnr1p and 7 μ g of Rnr2p/Rnr4p heterodimer) were incubated at 30 °C for 30 min in a final volume of 50 μ l: 25 nmol of [³H]CDP (specific activity 37000 cpm/nmol), 2 μ mol of Tris-HCl, pH 7.6, 0.125 μ mol of ATP, 0.32 μ mol of MgCl₂, 0.5 μ mol of dithiothreitol, 5 μ mol of KCl and 1 nmol of FeCl₃. In addition, the tubes contained increasing amounts of *Sml1p*. After incubation, the samples were processed as described earlier to obtain the amount of dCDP formed (22).

Rnr4p—The C-terminal sequence of the *Sml1p* shows some homology to the C-terminal peptides of the Rnr2 or Rnr4 proteins. Because such peptides are known to inhibit RNR activity by binding to the R1 protein and preventing R1/R2 interaction, we wanted to study the influence of a C-terminal nonapeptide of *Sml1p* on yeast RNR activity. As shown in Fig. 3, nonapeptides from the C terminus of Rnr2p or Rnr4p inhibited the *in vitro* yeast RNR assay to about the same extent with an IC_{50} of 44 and 30 μ M, respectively. In contrast, the nonapeptide corresponding to the C terminus of *Sml1p* showed an inhibition with an IC_{50} of only about 300 μ M.

Interaction between Yeast Rnr1 Protein and *Sml1* Protein Assayed by Sucrose Gradient Centrifugation—For enzymatic activity, ribonucleotide reductases of the class Ia-type must form a heterodimeric complex composed of homodimeric R1 and R2 proteins. It was previously demonstrated for the mouse RNR that binding of effectors to the substrate specificity site promotes formation of the R1 dimer, which is believed to be a prerequisite for binding to the R2 dimer (18, 23). To test if *Sml1p* binding to the Rnr1p might interfere with Rnr1p dimer formation and thereby inhibit RNR activity, we preincubated Rnr1p and *Sml1p* in the presence of the allosteric effector dTTP; we then analyzed the mixture on a sucrose gradient (Fig. 4). The addition of dTTP induced formation of dimers and tetramers of the Rnr1p, and no monomer peak could be seen in the gradient. This pattern is quite different from the one shown in the absence of dTTP, where the Rnr1p monomers dominate (Fig. 4). Addition of *Sml1p* did not influence the distribution of Rnr1p in the gradient, and no shift from dimers to monomers

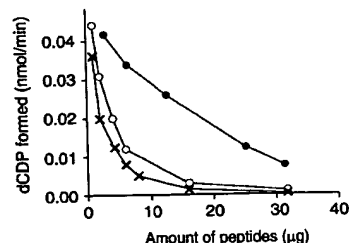


FIG. 3. Inhibition of yeast RNR by nonapeptides corresponding to the C-terminal ends of Sml1p, Rnr2p or Rnr4p. A series of tubes containing yeast RNR (10 μ g of Rnr1p and 1 μ g of Rnr2p-Rnr4p heterodimer) were incubated at 30 $^{\circ}$ C for 30 min as described in Fig. 2 in the presence of increasing amounts of nonapeptide. (●), Sml1 peptide; (○), Rnr2 peptide; ×, Rnr4 peptide.

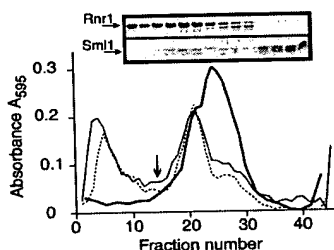


FIG. 4. Sucrose gradient centrifugation of yeast Rnr1p in the absence or in the presence of dTTP, with or without Sml1p. Sedimentation (from the right to the left) was performed as described under "Materials and Methods." Catalase ($s_{20,w} = 11.4$ S) sedimented in fraction 14 (arrow). Thick line, Rnr1 protein sedimented alone without dTTP or Sml1p. The two peaks represent the dimer (fraction 21) and monomer (fraction 25) positions in the gradient. Hatched line, Rnr1p sedimented in the presence of dTTP. Most of the material now sedimented as tetramers (fraction 5) or dimers (fraction 21). Note that the small peak sedimenting at fraction 26 represents an impurity in the Rnr1p preparation (the band just below the R1 band in the gel insert). The sedimentation of this material is not affected by dTTP. Thin line, Rnr1p with Sml1p sedimenting in the presence of dTTP. Insert, SDS-PAGE (15% gel) analysis of fractions from the gradient. Each lane on the gel is positioned above the corresponding fraction in the gradient.

could be observed. At the same time, analysis of the fractions by SDS-PAGE clearly demonstrated two peaks of Sml1p, one minor peak cosedimenting with the dimer peak of Rnr1p, and one major peak representing free Sml1p sedimenting at the top of the gradient. The fact that a portion of Sml1 protein was present in the fractions containing the Rnr1p dimers indicates that the Sml1p can bind to the Rnr1p dimer without dissociating it into monomers. No shift toward Rnr1p monomers was observed under the following conditions: the Rnr1p/Sml1p/dTTP incubation mixture was sedimented through a gradient containing Sml1p at a concentration of 0.15 mg/ml throughout to minimize Rnr1p-Sml1p dissociation (data not shown). Therefore, binding of Sml1p did not influence the monomer/dimer equilibrium of the Rnr1p.

Kinetic Studies of the Interaction between the Rnr1 and Sml1 Proteins Using a Biosensor Technique—To obtain a quantitative description of the Rnr1p and Sml1p interaction, we immobilized the Sml1 protein to the dextran layer of a sensor chip and then injected a series of solutions containing increasing concentrations of Rnr1p to the same sensor chip. The immobilization of Sml1p at a concentration of 0.44 mg/ml gave an increase of 91 RU, which correspond to 91 pg/mm². With a surface of 0.8 mm², 91 RU corresponds to a total of 72.8 pg of bound Sml1p, which can be compared with the total injected amount of 15.4 μ g. This low degree of attachment (only 0.0005%) makes it unlikely that the protein is attached to multiple binding sites.

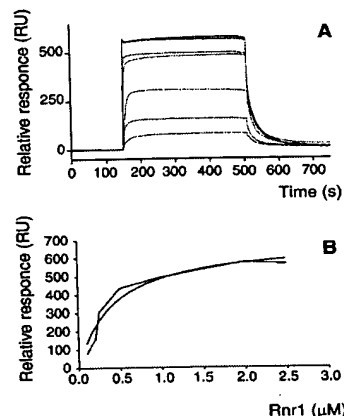


FIG. 5. A, sensorgram showing the interaction between immobilized Sml1p (91 RU) and increasing concentrations of mobile phase Rnr1p (0.1, 0.2, 0.25, 0.5, 1, 2, and 2.5 μ M using the monomer molecular weight of 100,000). Injection starts at 150 s and ends at 510 s. In this figure, the bulk effect response of around 60 RU has been subtracted. B, dose-response curve where the response at equilibrium is plotted against the concentration of Rnr1p (the same data as shown in A). The plot shows both the experimental curve and a curve fitted to a one-site binding model (hyperbola) by the GraphPad Prism program.

Increasing concentrations of Rnr1p resulted in increasing equilibrium values, which approached a maximal value (Fig. 5A). In control experiments, where the same series of Rnr1p solutions passed a sensor chip without immobilized Sml1p, a "bulk effect" gave a response of only about 60 RU. The same background value of about 60 RU was observed when bovine serum albumin or Rnr2p/Rnr4p heterodimer was injected at a concentration of 0.1 mg/ml (data not shown). In Fig. 5B, the response at equilibrium is plotted against the concentration of injected Rnr1p. Using the GraphPad Prism program (GraphPad Software, Inc.), an equilibrium dissociation constant (K_D) of 0.41 ± 0.1 μ M and a maximal binding of 691 RU were obtained. These values correspond to 691 pg/mm² or a total of 553 pg or 5.5 fmol of Rnr1p monomer bound to about 6.2 fmol of immobilized Sml1p, i.e. nearly one Rnr1p monomer bound per molecule of immobilized Sml1p. Using the BIAevaluation software (Biacore AB), an association rate constant of about $153,000$ M⁻¹ s⁻¹ and a dissociation rate constant of 0.04 s⁻¹ were obtained from the lower curves in Fig. 5A. Calculating the equilibrium dissociation constant from these rate constants gave a K_D of about 0.25 μ M, which is close to the directly determined K_D . Mixing the Rnr1p with Rnr2p/Rnr4p heterodimer, with or without dTTP before injection, did not affect the curves; the same results were obtained as when Rnr1p was injected alone.

Inhibition of Mouse Ribonucleotide Reductase by the Sml1 Protein—The effects of Sml1 protein on pure recombinant mouse ribonucleotide reductase were tested in a CDP assay in the presence of ATP as a positive effector (Fig. 6). In the figure, RNR activity in the presence of a fixed amount of R1 protein, and in the presence or absence of a 300-fold molar excess of Sml1p, are plotted against increasing concentrations of the R2 protein. In contrast to the situation with the yeast RNR, this inhibition is less pronounced and dependent on the R1 to R2 ratio. In a double reciprocal plot, clear competition is observed between Sml1p and the R2 protein (data not shown).

Kinetic Studies of the Interaction between the Mouse RNR R1 Protein and the Sml1 Protein Using a Biosensor Technique—After observing inhibition of the mouse RNR by Sml1p, we wanted to characterize the binding between the mouse R1 protein and Sml1p. As before, the Sml1p was immobilized on a

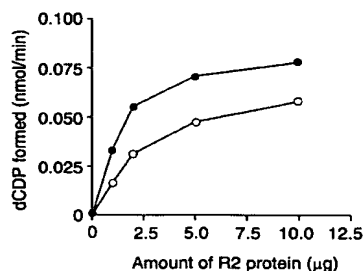


FIG. 6. Inhibition of mouse RNR by the Sml1p. A series of tubes containing mouse RNR (1 μ g of R1 protein and the indicated amounts of R2 protein) were incubated at 37 $^{\circ}$ C for 30 min as described in Fig. 2 in the absence (\bullet) or in the presence of 36 μ g of Sml1p (\circ).

sensor chip, and a solution containing 0.1 mg/ml of mouse R1 protein was injected (Fig. 7). On injection, a very rapid association phase was observed; this phase was immediately followed by a prolonged dissociation phase that never reached an equilibrium plateau. Injecting the R1 solution over an empty sensor chip gave the same low background value as observed earlier. Injection of mouse R2 protein also resulted in only background values (data not shown). To exclude the possibility that the unexpected behavior of the mouse R1 protein was due to improperly immobilized Sml1p, we injected 0.1 mg/ml of the yeast Rnr1 protein; we observed the same type of response as in Fig. 5A, with a clear equilibrium plateau. Knowing that allosteric effectors affect the conformation of R1 proteins, we next mixed the mouse R1 protein with dTTP before injection. This time, a rapid association phase was followed by a clear equilibrium plateau; this sensorgram resembled the sensorgram obtained with the yeast Rnr1p. Finally, we mixed the mouse R1 protein plus dTTP with R2 protein before injection, expecting to see an R1-R2 complex bound to Sml1p. However, the R2 protein addition almost abolished the specific R1 binding, and resulted in almost background values. No attempts were made to quantify the mouse R1 binding data, because the sensorgrams deviated widely from standard curves.

DISCUSSION

Our *in vitro* data directly prove the hypothesis of Zhao *et al.* (13) that the yeast Sml1 protein is a physiological inhibitor of ribonucleotide reductase; this is a new concept in the RNR field. So far, no mammalian homologue has been identified, and no homologous proteins could be found in available data bases. This situation may reflect different control of ribonucleotide reductase activity in yeast and mammalian cells. In yeast, transcriptional activation of the RNR genes and suppression of the Sml1 protein lead to increased RNR activity and deoxyribonucleotide pools after DNA damage (12, 13). In contrast, mammalian cells control RNR activity by an S-phase/DNA-damage specific stabilization of the R2 protein, until cells enter into mitosis, in combination with negative feedback regulation by dATP². We suggest that the Sml1 protein may substitute for dATP feedback regulation, since yeast RNR is not inhibited by physiological concentrations of dATP. Therefore, unlike in mammalian cells, there is a direct correlation between levels of RNR proteins and deoxyribonucleotide pools in yeast cells.

The Sml1 protein specifically binds to the yeast Rnr1 protein with a dissociation constant of 0.4 μ M and in a 1:1 ratio at saturation. Binding did not influence the R1 monomer/dimer equilibrium, and no competition with the R2-R4 heterodimer was observed. These observations may indicate separate binding areas on the R1 protein, or that the affinity of the Rnr1p/Sml1p interaction is much higher compared with the Rnr1p interaction with the R2/R4 heterodimer. For mouse and *E. coli*

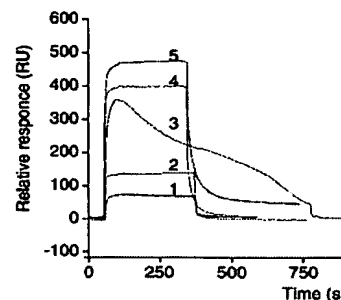


FIG. 7. Sensorgram showing the interaction between immobilized Sml1p and the mouse R1 protein. Curve 1 shows the bulk effect response when R1 protein (0.1 mg/ml) is passed over a sensor chip without Sml1p. Curve 2 shows the response when a mixture of mouse R1 and R2 proteins (both at 0.1 mg/ml) in the presence of 0.1 mM dTTP is passed over a sensor chip with immobilized Sml1p. Curve 3 shows the response with mouse R1 protein alone at 0.1 mg/ml passed over immobilized Sml1p. Note that the injection time for this curve is prolonged from 6 to 14 min. Curve 4 was obtained with mouse R1 protein at 0.1 mg/ml in the presence of 0.1 mM dTTP passed over immobilized Sml1p, and Curve 5 shows the response obtained with yeast Rnr1p at 0.1 mg/ml passed over immobilized Sml1p.

RNRs, the dissociation constant for the R1-R2 complex was reported to be around 0.1 μ M (18); the subunit interaction in the yeast RNR appears much weaker, however, and we have not yet been able to quantify it. On a molar basis, the Sml1 protein is about 200 times more efficient in inhibiting yeast RNR activity than its C-terminal nonapeptide. Furthermore, unlike Sml1p inhibition, peptide inhibition depends on the concentration of the R2-R4 heterodimer.

The yeast Rnr1 protein and the mouse R1 protein show 68% amino acid sequence identity, and the sequence similarities are distributed along the whole polypeptide chain. Still, it was quite unexpected that the Sml1 protein would also specifically bind to the mouse R1 protein. However, as indicated by the sensorgram that shows mouse R1 binding to Sml1p, the initial rapid binding was followed by a prolonged dissociation that is prevented by the addition of dTTP. This finding may indicate conformational changes induced by dTTP that makes the R1 protein a better target, or that binding of the R1 dimer is preferred. Furthermore, both in the mouse RNR assay and in the biosensor experiments, clear competition was observed between Sml1p and the mouse R2 protein for binding to the R1 protein. Therefore, the inhibition mechanism may differ for yeast and mouse RNR enzymes. Although inhibition in the yeast system is very efficient and may involve blocking the entrance to the active site, inhibition in the mouse system is rather inefficient and may reflect blocking R2 protein binding to the R1 protein, perhaps in the same way as R2 protein C-terminal peptides.

We hope that structural studies of the Sml1 protein, alone and bound to the R1 protein will reveal the mechanism of inhibition. This finding should be useful in developing specific antiproliferative inhibitors of RNR; such new inhibitors could complement existing ones, which include radical scavengers, iron chelators, and peptidomimetics (2).

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